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BIOCHEMICAL DIFFERENTIATION IN THE IDAHO GROUND SQUIRREL, *SPERMOPHILUS BRUNNEUS* (RODENTIA: SCIURIDAE)

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ABSTRACT.—*Spermophilus brunneus* is restricted to a 90 × 125-km area of west central Idaho, with two distinct (northern and southern) groups of populations within this limited range. Morphological differences in pelage length and coloration, external and cranial measurements, and bacula suggest that these groups are either very distinct subspecies or species. We used starch-gel electrophoresis to estimate the amount of genetic differentiation accompanying these morphological differences by assaying genetic variation at 31 loci in the two geographic groups. Fifteen loci were polymorphic (13 in the northern group, 12 in the southern), and mean heterozygosity ($\bar{H}$) was high (12.3% northern and 10.8% southern). Nei's genetic distance (0.057) is in the range usually associated with subspecific differences. However, Jaccard's association coefficient (0.893) is about the same as that found between several ground squirrel taxa currently recognized as species. The high levels of heterozygosity suggest that *S. brunneus* is a neoendemic rather than a paleoendemic species.

Key words: Spermophilus brunneus, Spermophilus, *Idaho ground squirrel, ground squirrels, electrophoresis, taxonomy, biochemical differentiation.*

Spermophilus brunneus is one of the rarest, least studied, and most geographically restricted of the North American ground squirrels. Within its restricted range of ca 90 × 125 km in west central Idaho there are two well-differentiated subspecies, *S. b. brunneus* and *S. b. endemicus* (Yensen 1991). Significant differences in pelage length and color, external and cranial measurements, and bacular morphology suggest that the two taxa may be close to species-level separation (Yensen 1991). The northern *Spermophilus b. brunneus* is known from only ca 20 isolated sites in mountain meadows in Adams and Valley counties. These demes consist of <200 individuals and are separated from each other by distances of 1–70 km. In contrast, the southern *S. b. endemicus* is patchily distributed over a contiguous area 70 km long and up to 20 km wide in the lower-elevation foothills of Gem, Payette, and Washington counties (Yensen 1991).

Davis (1939) divided the North American species of subgenus *Spermophilus* into "small-eared" and "large-eared" groups and placed *S. brunneus* within the large-eared group. Nadler et al. (1973) found, however, that the karyotypes of *S. brunneus* and *S. townsendii mollis* (small-eared group) differed only in the presence or staining intensity of minor bands on six chromosomes, indicating a close affinity between *S. brunneus* and the small-eared group. Nadler et al. (1974) analyzed serum transferrins of *S. brunneus* using starch-gel electrophoresis and concluded that it was biochemically "intermediate" and possibly ancestral to both the Nearctic "small-eared" and "big-eared" species groups of subgenus *Spermophilus*. Nadler et al. (1982) extended their analysis to 21 Holarctic species using 18 loci and concluded that *S. brunneus* was a paleoendemic species most closely related to the Eurasian *S. dauricus*. Nadler et al. (1984) revised their phylogeny to incorporate chromosomal data and placed the evolutionarily conservative *S. brunneus* within the *S. townsendii* group.

The present study was conducted to estimate the genetic differentiation accompanying the substantial morphological differences between the two geographic groups of *S. brunneus* and to assess the hypothesis that *S. brunneus* is a paleoendemic species with small, relictual populations.

MATERIALS AND METHODS

Specimens Analyzed

A total of 82 specimens were analyzed from the following localities: *Spermophilus brunneus*

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brunneus—Adams Co.: 1 mi NE Bear Guard Station, 3; Bear Cemetery, 2; Cold Springs Cr., 1; Little Mud Cr., 5; Mill Cr. 3 mi N Hornet Guard Station, 2; New Meadows, 12; Price Valley, 2; Lick Cr., 6; Summit Cr., 9. *Spermophilus brunneus endemicus*—Gem Co.: Sucker Cr. 11 mi N Emmett, 20; 12.6 mi N Emmett, 1; Payette Co.: Big Willow Cr., 1; Dry Cr. Road, 3; Washington Co.: Lower Mann Cr., 10; Weiser Cove, 5. These specimens have been deposited as vouchers in the Albertson College Museum of Natural History.

Laboratory Methods

Blood was collected from the suborbital sinus of living animals (samples sizes were 21 *S. b. brunneus*, 9 *S. b. endemicus*). Liver and kidney tissues were from sacrificed animals (10 *S. b. brunneus*, 6 *S. b. endemicus*) or frozen carcasses collected for other purposes (18 *S. b. brunneus*, 31 *S. b. endemicus*). Carcasses were stored at −20 C for 1–6 months.

Tissue sample preparation and horizontal starch-gel electrophoresis follow Selander et al. (1971) with slight modifications. We used 11.0% electrostarch for lithium hydroxide gels and 12.4% for all other gels. Enzyme locus designations follow standardized Enzyme Commission (E.C.) nomenclature (Harris and Hopkinson 1976). The enzymes and nonenzymatic proteins screened in this study, with tissue and buffer systems used, were: alcohol dehydrogenase, E.C. No. 1.1.1.1 (ADH), liver, tris-citrate, pH 8.0; glycerol-3-phosphate dehydrogenase, E.C. No. 1.1.1.8 (GPD), liver, tris-citrate, pH 8.0; L-iditol dehydrogenase, E.C. No. 1.1.1.14 (IDDH), liver, tris-citrate, pH 8.0; lactate dehydrogenase, E.C. No. 1.1.1.27 (LDH), kidney, tris-citrate, pH 8.0; malate dehydrogenase, E.C. No. 1.1.1.37 (MDH), liver, tris-citrate, pH 6.3; isocitrate dehydrogenase, E.C. No. 1.1.1.42 (ICD), kidney, tris-citrate, pH 8.0; superoxide dismutase, E.C. No. 1.15.1.1 (SOD), kidney, tris-maleate or tris-citrate, pH 8.0; aspartate aminotransferase, E.C. No. 2.6.1.1 (AAT), liver, lithium hydroxide; hexokinase, E.C. No. 2.7.1.1 (HK), kidney, tris-citrate, pH 8.0; phosphoglucomutase, E.C. No. 2.7.5.1 (PGM), kidney, tris-citrate, pH 8.0; esterase, E.C. No. 3.1.1.1 (ES), hemolysate, tris-hydrochloric acid; peptidase, E.C. No. 3.4.11 or 13.* (PEP), liver, tris-citrate, pH 6.3; hemoglobin (HGB), hemolysate, tris-hydrochloric acid; albumin (ALB), plasma, lithium hydroxide; transferrin (TRF), plasma, lithium hydroxide; general proteins (GP1 and GP2), hemolysate, tris-hydrochloric acid; and general proteins (GP3 and GP4), plasma, tris-hydrochloric acid. The proteins were numbered in order of decreasing mobility, with the most anodal labeled 1.

The buffer and stain systems for the proteins screened in this study were described by Selander et al. (1971), except for stains for IDDH, HK, and PEP (Gill et al. 1987). Of the esterases, only acetylesterases were stained and were numbered 1 (most anodal) to 5. PEP-C was detected with L-leucyl-L-alanine. ADH does not have to be stained specifically and is seen on many dehydrogenase gels. It was read on gels stained for GPD.

Computational Methods

Gene frequencies, measures of genetic variation, Nei's (1978) unbiased genetic distance and unbiased genetic identity, and the average inbreeding coefficient (F_{ST}) were derived from input on single individual genotypes (electromorphs) using the computer program BIOSYS-1 (Swofford and Selander 1981). Jaccard's association coefficient, $S_j = a/(a+u)$, where a = the number of matched electromorphs (1:1) and u = the number mismatched (1:0 or 0:1) (Sneath and Sokal 1973), was also calculated for the two groups. S_j depends only upon the presence (1) or absence (0) of alleles, as indicated by bands on the starch gels (electromorphs), not on allelic frequencies as do measures of genetic distance. Negative matches were excluded.

Results and Discussion

Spermophilus b. brunneus was polymorphic at 13 loci (42%), whereas *S. b. endemicus* was polymorphic at 12 loci (39%). If esterases are excluded, polymorphism is reduced to 31%, which is similar to the 29% reported for *Mus musculus* and *Homo sapiens* (Lewontin 1974). Average number of alleles per locus ($\bar{A}$) was 1.48 ± 0.11 ($\bar{X}$ ± SE) in *S. b. brunneus* and 1.48 ± 0.12 in *S. b. endemicus*. All polymorphic loci had two alleles, except for peptidase and two of the esterases, which had three.

Mean heterozygosity per individual per locus in our sample was 12.3 ± 3.7% in *S. b. brunneus* and 10.8 ± 3.9% in *S. b. endemicus*. These values are much higher than the 2.7% heterozygosity reported by Nadler et al. (1982)

for *S. b. brunneus*. The loci common to both studies, however, were less variable than some of our 18 additional loci. Even if esterases are excluded from the analysis, our measures of genetic variability (*S. b. brunneus*, $\overline{H} = 8.2\%$, $\overline{A} = 1.35$; *S. b. endemicus*, $\overline{H} = 7.4\%$, $\overline{A} = 1.38$) are still much higher than theirs. They found $\overline{H}$ values of 0.0–10.4% ($\overline{X} = 3.5\%$) for other species of *Spermophilus*. Cothran et al. (1977) found high heterozygosity (9.3%) in the ground squirrel subgenus *Ictidomys*. The average heterozygosity for 26 taxa of rodents was 5.4% (Selander 1975), so Idaho ground squirrels have relatively high levels of heterozygosity. Thus, the levels of genetic variability are high for a species postulated to be a paleoendemic (Nadler et al. 1974, Cothran et al. 1977, Nadler et al. 1982) with small isolated demes and confined to a small geographic area (Yensen 1991).

Sixteen of 31 protein systems scored for *S. brunneus* were monomorphic (GPD, LDH-A, ICD-2, HK-1,2, PGM-1,2, AAT-1,2, IDDH, SOD-B, ADH, ALB, TRF, GP-1,2). Frequencies of alleles in the polymorphic systems (the most common allele <0.99) are shown in Table 1. As in other species (Kojima et al. 1970, Lewontin 1974), non-glucose–metabolizing enzymes were more polymorphic than glucose-metabolizing enzymes with five monomorphic (AAT-1,2, IDDH, SOD-B, and ADH), while PEP-C, SOD-A and all five esterases were polymorphic (Table 1). The two taxa of *S. brunneus* did not differ substantially in glucose-metabolizing enzymes, with the majority of loci monomorphic, and the same allele common in the polymorphic loci.

Nadler et al. (1982) found LDH to be monomorphic in all 21 North American and Eurasian *Spermophilus* species examined. However, we found two individuals of *S. b. brunneus* that were homozygous for a fast allele at the LDH-B locus. Nadler et al. (1982) assayed from LDH in red blood cells while we used kidney extracts, so the difference may be between the two tissues. Both groups of *S. brunneus* were polymorphic for ICD-1 and HK-3, while only *S. b. endemicus* was polymorphic for MDH-1.

Of the enzymes not involved in glucose metabolism, the esterases were the most variable (Table 1). We also found considerable differences between *S. b. brunneus* and *S. b. endemicus* in the other non-glucose–metabolizing enzymes. Different alleles were common for PEP-C and ES-4 in the two groups of *S. brunneus*. In both cases the differences were in allelic frequency rather than in the presence or absence of alleles.

TABLE 1. Allelic frequencies of polymorphic loci in *Spermophilus brunneus*.

Locus*	Allele**	*brunneus*	*endemicus*
GLUCOSE-METABOLIZING ENZYMES			
LDH-B	a	0.929	1.000
	b	0.071	0.000
MDH-1	a	0.000	0.018
	b	1.000	0.911
	c	0.000	0.071
ICD-1	a	0.926	0.986
	b	0.074	0.014
HK-3	a	0.132	0.097
	b	0.868	0.903
NON-GLUCOSE-METABOLIZING ENZYMES:			
SOD-A	a	0.786	0.957
	b	0.214	0.043
PEP-C	a	0.365	0.329
	b	0.135	0.343
	c	0.500	0.329
ES-1	a	0.179	0.056
	b	0.107	0.167
	c	0.714	0.778
ES-2	a	0.969	1.000
	b	0.031	0.000
ES-3	a	0.971	0.944
	b	0.000	0.056
	c	0.029	0.000
ES-4	a	0.714	0.389
	b	0.286	0.611
ES-5	a	0.656	0.944
	b	0.344	0.056
NONENZYMATIC PROTEINS.			
HGB-1	a	0.233	0.667
	b	0.767	0.333
HGB-2	a	0.100	0.500
	b	0.900	0.500
GP-3	a	0.000	1.000
	b	1.000	0.000
GP-4	a	0.962	0.750
	b	0.038	0.250

*See text for acronyms of loci.
**Alleles are listed in order of increasing mobility; a is slowest

Nonenzymatic proteins were scored in both hemolysate and plasma. Albumin and transferrin in plasma and two general proteins in hemolysate were monomorphic. We found variability at the two hemoglobin loci and at two general protein loci in plasma (Table 1). Heterozygosity of hemoglobins has been found in the closely related Townsend's ground squirrel (*S. townsendii*), in which the two hemoglobins have identical α-chains and differ by only one amino acid in the sequence of their β-chains

(Kleinschmidt et al. 1985). They found no difference in the oxygen affinity of the two hemoglobins.

A general protein in plasma (GP-3) represented by a band just anodal to albumin distinguished the two *S. brunneus*. A fast allele apparently has reached fixation in *S. b. brunneus*, whereas a slow allele appears fixed in *S. b. endemicus* (Table 1). This is the only locus that can serve as a marker gene among the 31 loci scored, although LDH-B and MDH-1 had alleles that were fixed in one taxon and polymorphic in the other. The other presumed loci differed in allelic frequency only.

Nei's (1978) genetic distance is a measure of the accumulated number of gene differences per locus between populations. The genetic distance of 0.057 found between the two *S. brunneus* was within the range associated with subspecific differentiation (Avise 1974). The average inbreeding coefficient (F_{ST} = 0.167) indicated moderately high genetic differentiation. The two *S. brunneus* have a genetic identity of 0.944. By comparison, Cothran et al. (1977) found genetic identities of 0.808 between *S. spilosoma* and *S. mexicanus*, 0.835 between *S. spilosoma* and *S. tridecemlineatus*, and 0.965 between *S. tridecemlineatus* and *S. mexicanus* in the subgenus *Ictidomys*.

To compare our results with other results from the subgenus *Spermophilus* (Nadler et al. 1982), we also calculated Jaccard's association coefficient. This measure is less sensitive to sample size and depends on presence or absence of an allele, rather than on allelic frequencies. Jaccard's coefficient of similarity between the two groups of *S. brunneus* was 0.893. Judging from Figure 2 in Nadler et al. (1982:206), the similarity between the two groups of *S. brunneus* is about the same as the similarity between *S. armatus* and *S. beldingi*, or between some of the putative semispecies in the *S. townsendii* complex, the Eurasian *S. suslicus* and *S. citellus*, or *S. major* and *S. erythrogenys*. *Spermophilus richardsoni* and *S. elegans* are more similar electrophoretically than the two Idaho ground squirrels. However, direct comparisons are difficult since the similarity coefficients computed by Nadler et al. (1982) were based on a different, and apparently less variable, set of loci.

The electrophoretic data confirm that the two Idaho ground squirrels are genetically as well as morphologically differentiated taxa. The evidence does not clearly resolve the question of whether the two are separated at the subspecies or species level. The presence of one marker gene and the observed frequency differences at others could be consistent with either interpretation. The high levels of heterozygosity, however, do not support the paleoendemic hypothesis.

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